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AN ANATOMICAL STUDY OF THE RETINA OF *NAUTILUS POMPILIUS*

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ABSTRACT

The receptor outer segments of *Nautilus* have several rhabdomeres of parallel microvilli occurring more or less equally at all orientations. The microvilli of adjacent receptors overlap substantially. The supporting cells of the retina possess both microvillous processes and small cilia. The receptor cells become disorganized towards their tips, although supporting cell processes can still be distinguished. There is no limiting membrane. Similarities to and differences from the retinas of adult and embryonic coleoid cephalopods are discussed.

The myeloid body of *Nautilus* is more developed than in other cephalopods, appearing in electronmicrographs as a complex of loops or circles. A series of dimpled plates stacked in register one above the other could generate most or all of these apparently complicated structures depending on the plane of section. Myeloid bodies occur both externally and internally to the nucleus, and occasionally within the outer segment.

At its margin the retina merges smoothly with the columnar epithelium lining the inside of the iris. The margin of the retina contains relatively more supporting cells than more central regions. Myeloid bodies first appear about 200 μm central to the point at which the retina starts to thicken.

INTRODUCTION

The Nautiloidea, a highly successful group of cephalopods which originated in the Cambrian period, are today only represented by a few species of *Nautilus* which are restricted to deep waters in tropical areas of the Pacific and possibly the Indian oceans. *Nautilus* differs in many respects from the more recent coleoid cephalopods and, although undoubtedly specialized in many ways for its deep-water habitat (Landman and Saunders, 1987), shows many characteristics that appear to have remained unchanged since Jurassic times. Therefore, its study is of special interest in that it may provide some insight into how the organization of the modern cephalopods arose.

The visual system is one of the features that may be primitive in *Nautilus*. The eye is structurally simple, consisting of a cup formed by an invagination of skin, communicating directly with the surrounding water by way of the open pupil. The outer and inner surfaces of the iris are lined with columnar epithelium bearing microvilli, which on the inside merges through a transitional zone with the retina itself: as in other cephalopods, the rhabdomeres of the outer segments of the retinal receptors are formed from modified microvilli (Messenger, 1981). The inner segments of the receptors contain nuclei and myeloid bodies [Merton's (1905) "phaosomes"]. The latter, which are probably concerned with visual pigment metabolism (Hara and

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Hara, 1972; Breneman *et al.,* 1986), are especially interesting because they are much more structurally complex than those found in the more recent coleoid cephalopods.

The structure of the eye has been the subject of a number of histological studies at both the light and the electron microscope levels (*e.g.,* Hensen, 1865; Griffin, 1900; Owen, 1932; Mugglin, 1937; Young, 1965; Barber and Wright, 1969; Muntz and Raj, 1984). Behavioral studies have also shown, as would be expected in an eye that has no lens but operates on the principle of a pin-hole camera, that both visual acuity and sensitivity are poor in comparison to equivalent lens-bearing eyes (Muntz and Raj, 1984; Muntz, 1987).

The present paper presents further information on various aspects of retinal structure: in particular on the interrelationship between the rhabdomeres of neighboring receptors and the supporting cells, the transitional zone between the epithelium lining the inside of the iris and the retina, and the structure of the supporting cell processes and the myeloid bodies.

MATERIALS AND METHODS

Specimens of *Nautilus* were trapped off the main reef at Suva, at depths between 165 and 600 m, and kept in glass aquaria in an air conditioned room at water temperatures between 7 and 13°C. The water was changed regularly, and the animals fed occasionally on prawns. Under these conditions they remained alive for several weeks, and one animal showed reliable positive phototactic behavior after more than 11 months in the laboratory. However histological preparations show some degeneration of the retina after a prolonged period in the laboratory, and the optomotor response also becomes less reliable (Muntz and Raj, 1984). Therefore the present paper reports results on animals used within 3 or 4 days of capture.

Small pieces of iris and retina were fixed in 2% glutaraldehyde made up in either Sörensen's phosphate buffer or filtered seawater. After 1.5 h fixation the tissue was washed in several changes of buffer or seawater as appropriate. The tissue was then post-fixed in 1% osmium tetroxide for 1 h. In some cases this post-fixation was done in Fiji and the tissue taken through to 70% ethanol before being brought back for further treatment. In most cases, however, the tissue was stored in fresh buffer or seawater and the further treatment, including post-fixation, carried out at Stirling or Monash University. Following post-fixation the tissue was either block stained in 2% uranyl acetate for 1 h or immediately dehydrated. Either Emix (EMscope, Ashford, England) or Spurr's resin (Spurr, 1969) were used to embed the tissue. Blocks were sectioned on either a Reichert 0M3 Ultratome or an LKB "Ultratome III." Thin sections (2–4 μm) were taken and stained with Toluidine Blue (pH 9.0) and examined under the light microscope. Ultrathin sections were stained with a saturated solution of uranyl acetate in 50% methanol and post-stained in lead citrate. The sections were examined using a JEOL JM100C(X) or an AEI Corinth 275 electron microscope.

In this paper the words inner or internally, when applied to the retina, mean towards the center of the animal, while outer or externally mean in the opposite direction, that is towards the light.

RESULTS

Outer segments and supporting cells

Figures 1a and b show electron micrographs of sagittal sections through the outer segments and supporting cells of the retina, confirming the structure described by Barber and Wright (1969). The tubules or microvilli that make up the rhabdomeres can be seen leaving the outer segments, and where the plane of section cuts across the microvilli they appear, as in other cephalopods (*e.g.*, Cohen, 1973), to be closely packed. The length of the microvilli varies from 2 to 6 μm between different rhabdomeres, but within a given rhabdomere their length does not change appreciably with position. The rhabdomeres are separated from each other by the supporting cells which contain a number of parallel processes extending over the whole depth of the outer segment layer of the retina (300–500 μm) and which, from their appearance in both transverse and longitudinal section, are clearly microvillar in nature. Figure 1b shows the origin of the supporting cell processes at the cell body. Small primary cilia are also found in the supporting cells (Fig. 3). It is not clear if they also occur in the receptor cells.

A tangential section through the outer segments is shown in Figure 2. The rhabdomeres radiate between the outer segments of neighboring receptors, separated by the processes of the supporting cells. The microvilli from neighboring receptors interdigitate, individual microvilli passing most, or perhaps all, of the way from one outer segment to another. Supporting cells have on average 46 processes (SD = 9, n = 30): on three occasions the processes from 2 or 3 supporting cells have apparently run together resulting in groups of about 90 or 150 processes.

Figure 4 shows, at the light microscope level, a sagittal section through the edge of the retina and the transitional zone between the retina and the iris. It can be seen that over about the outer third of their length the structure of the receptors becomes disorganized. Figure 5 shows the appearance of the outer region, near the receptor tips, as seen under the electron microscope. Processes belonging to the supporting cells can be seen, but the structure of the receptors themselves is no longer clearly organized into receptor cell bodies and rhabdomeres containing parallel microvilli. There is no sign of a limiting membrane overlying the outer ends of the receptors such as occurs in the coleoid cephalopods (Zonana, 1961; Yamamoto *et al.*, 1965).

Transitional zone between iris and retina

Figure 4 shows that at the junction of the iris and the retina there is a smooth increase in the thickness of the outer layer of the retina due to the increasing length of the receptor outer segments or supporting cell processes. The myeloid bodies do not start to occur at the point at which the retina thickens, but considerably further in: thus in preparations from four animals the distance from the first myeloid body to the edge of the retina (as indicated by the arrows in Fig. 4) ranged from 130 μm to 240 μm (mean 193 μm).

The edge of the retina also contains relatively few receptor cells relative to supporting cells compared to more central regions (Fig. 6). It is likely that the outer layers of the retina take on their final form at the same point as the myeloid bodies appear.

Structure of the myeloid bodies

The appearance of the myeloid bodies under the transmission electron microscope varied widely from a simple appearance of a number of wavy membranous structures, to a complex of loops, whorls, and circles (Figs. 7, 8, 9). The myeloid body is usually located in the inner segment of the receptors, although displaced myeloid bodies lying in the outer segment occasionally are found. In electron micrographs of 52 receptor inner segments in which both the myeloid body and the nucleus could be seen, the myeloid body lay internally to the nucleus on 33 occasions and externally on 20 occasions. The fine membranous structure of the myeloid body is clearly shown in Figures 8 and 9.

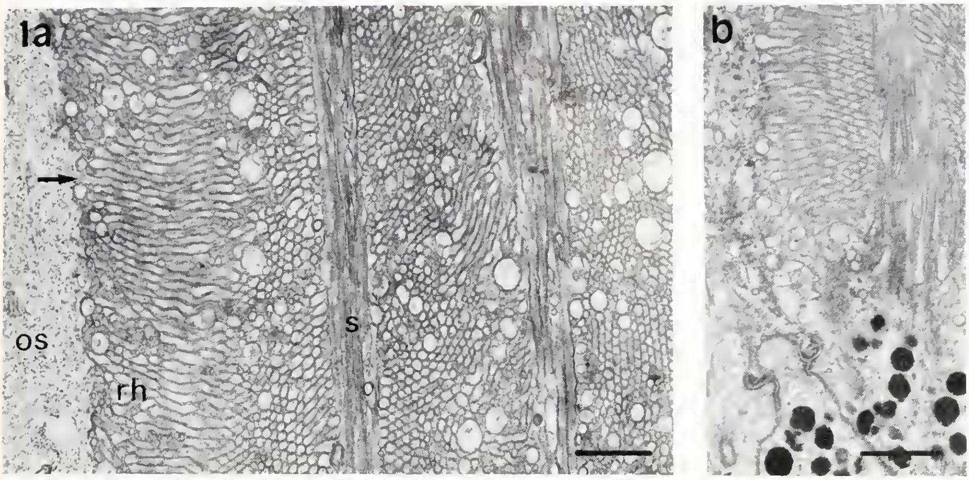
DISCUSSION

In the coleoid cephalopods that have been studied to date the arrangement of the retina as seen in tangential section is very precise. The basic plan, first described by Schultze (1869), is that each receptor outer segment carries a rhabdomere on two opposite faces, and that the rhabdomeres from four receptors combine to make a square rhabdome (Young, 1962)—originally thought by analogy with the anthropods to be the retina's basic functional unit. As a result of this structure the microvilli of the rhabdomeres form two orthogonal sets, oriented in the vertical and horizontal planes, respectively. There are some exceptions, and individual receptors can on occasion contribute to up to eight rhabdomeres (Zonana, 1961; Cohen, 1973), but the underlying plan is clear. The rhabdomeres of adjacent receptors remain separate, without any overlap in their microvilli. In contrast, each receptor outer segment in *Nautilus* has several rhabdomeres occurring more or less equally at all angles, and with substantial or complete overlap between the microvilli of neighboring receptors. There is no obvious regular grouping of the rhabdomeres from neighboring receptors to form a structure analogous to the rhabdome.

The orthogonal arrangement of the rhabdomeres of coleoid cephalopods, and the parallel relationship of the microvilli within them, is commonly assumed to underlie the ability of cephalopods to discriminate the plane of polarization of light (*e.g.*, Saidel *et al.*, 1983). The orthogonal arrangement does not occur in *Nautilus*, but the microvilli remain parallel to each other within any given rhabdomere, and the angular separation between each rhabdomere is still considerable even though less than 90°. Plane polarized light should thus still be able to affect the receptors of *Nautilus* differentially, but without any precise or predetermined pattern between the plane of polarization and the set of receptors that would be affected.

The interdigitation of the microvilli of neighboring receptors shown in Figure 2 is also visible in the electron micrographs published by Barber and Wright (1969), although not mentioned in that paper. Such interdigitation of microvilli is not unique to *Nautilus*, but occurs, for example, in some arachnids [*e.g.*, *Lampona* (Gnaphosidae), Blest, 1985] and is an arrangement that might be expected to affect visual acuity. Thus the simplest hypothesis relating retinal structure to acuity is that two objects will be discriminable when their retinal images are far enough apart that at least one receptor remains unstimulated between them (Helmholtz, 1924). Since microvilli overlapping will increase the separation between stimuli necessary to achieve this, a loss of resolution at the retinal level will result. However, in *Nautilus* the pin-hole arrangement for image formation appears to be the limiting factor for visual resolution with the retinal mosaic considerably finer than is needed for the quality of the image that is formed (Muntz and Raj, 1984). The overlapping of the microvilli, even if this halved the effective fineness of the retinal mosaic, should not affect the animal's ability to resolve detail. However, the overlap could benefit sensitivity, important for an animal living at depth in the sea, if sensitivity is limited to some degree by random retinal events that are unrelated to the stimulus, such as the spontaneous breakdown of visual pigment molecules or the spontaneous release of transmitters at synapses (visual "noise"). Overlap will increase the number of receptors simultaneously activated by a given stimulus, whereas "noise" events will presumably be independent in time between the different receptors. Provided the central nervous system is able to detect the simultaneity of messages from the receptors, sensitivity should be improved.

The retina of *Nautilus* also differs from that of the coleoid cephalopods in that the supporting cells have small processes that are ciliary in nature, as well as the microvillous processes extending between the receptors. Ciliary structures have not hitherto



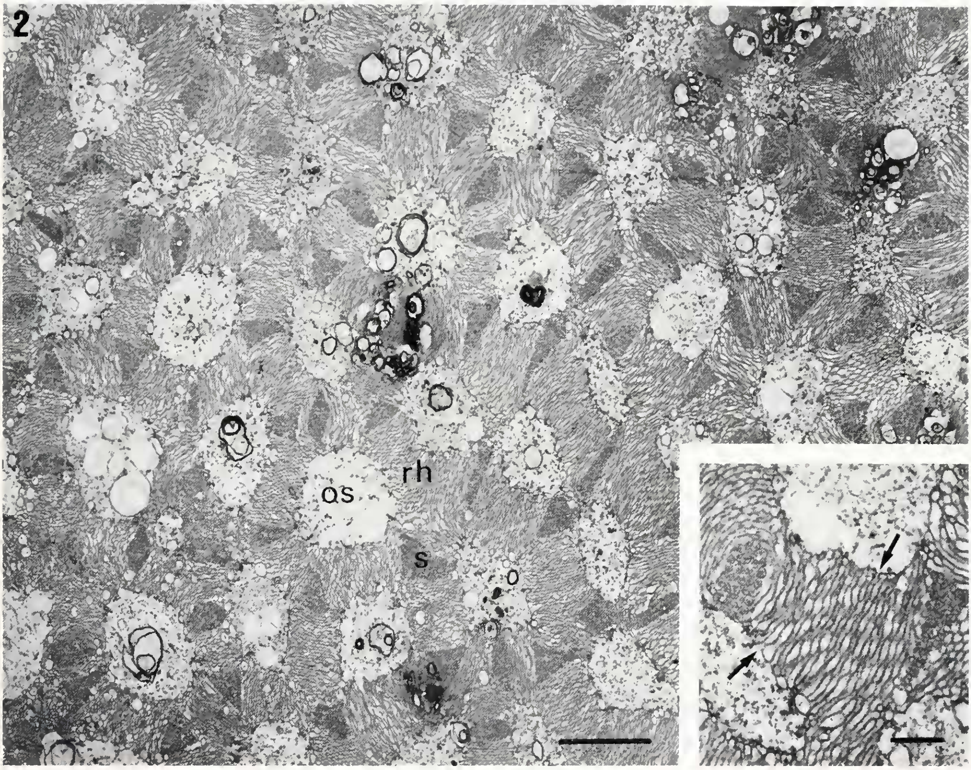
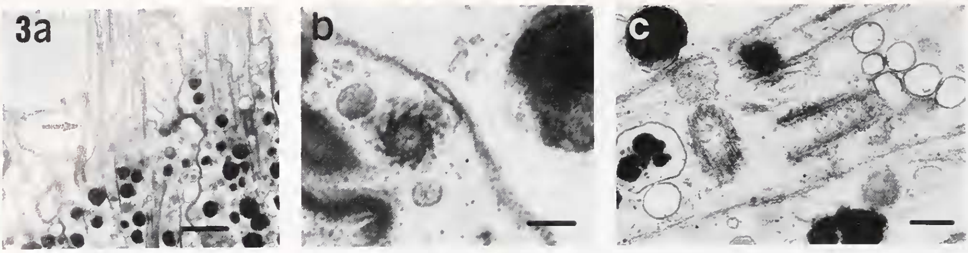
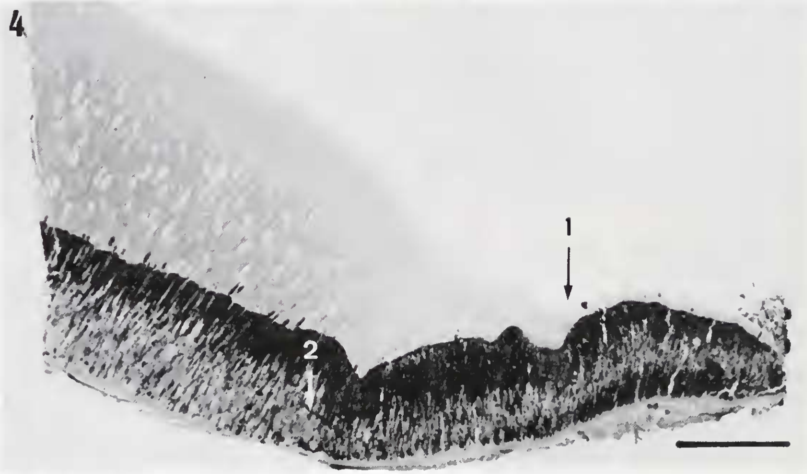


FIGURE 1. a. TEM of a sagittal section through the outer segments and supporting cells of the retina of *Nautilus pompilius.* os: outer segment, rh: rhabdomere, s: supporting cell. The arrow shows a microvillus opening into the outer segment. Fixative: 2% glutaraldehyde in seawater. Scale bar = 1 μm. b. TEM of a sagittal section of the receptor and supporting cells showing the origin of the supporting cell processes and receptor microvilli. Fixative: 2% glutaraldehyde in seawater. Scale bar = 1 μm.

FIGURE 2. TEM of a tangential section through outer segments and supporting cells of the retina. Labels as in Figure 1. Scale bar = 2 μm. The insert shows the appearance at higher magnification. The arrows show interdigitating microvilli from neighboring receptors. Fixative: 2% glutaraldehyde in seawater. Scale bar = 1 μm.





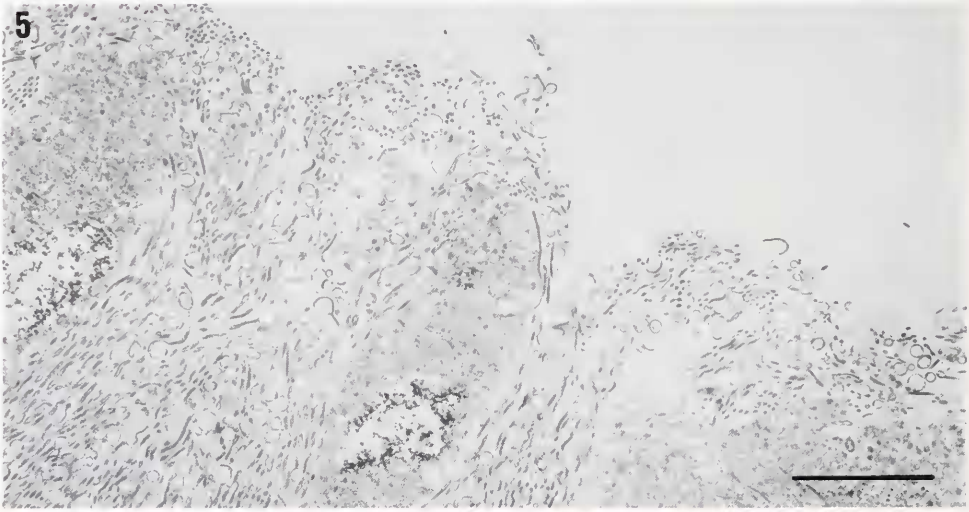
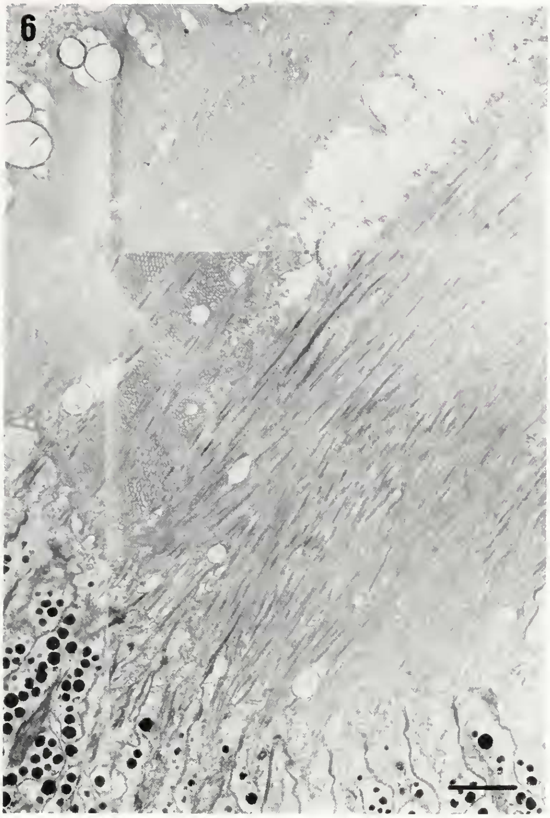
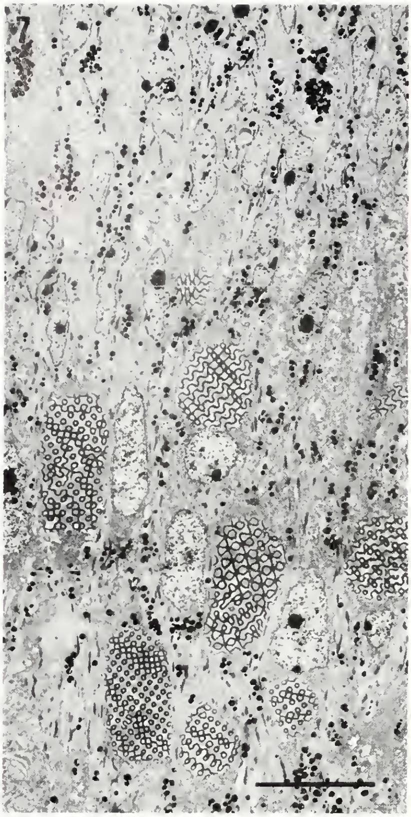


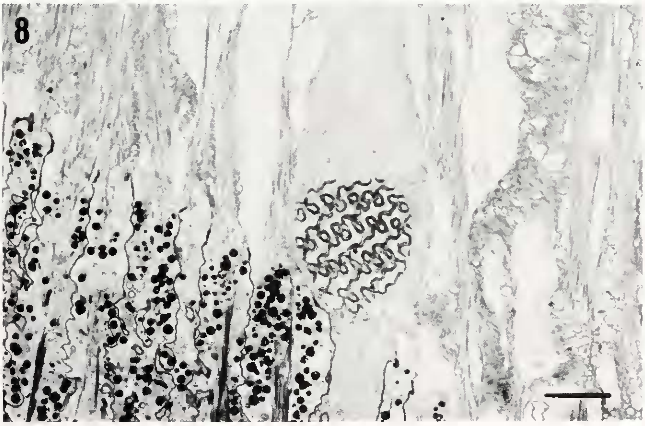
FIGURE 3. TEMs showing primary cilia in supporting cells and associated centriolar structures. Fixative: 2% glutaraldehyde in phosphate buffer. (a) Sagittal section of the retina. The cilium is indicated by the arrow. Scale bar = 1 μm. (b) Transverse section of the basal body of a cilium showing pinwheel structure. Scale bar = 0.2 μm. (c) Sagittal section of the retina showing diplosomal centrioles cut both longitudinally and obliquely. Scale bar = 0.2 μm.

FIGURE 4. LM of a sagittal section through the edge of the retina. The arrows show (1) the point at which the outer segments start to lengthen and (2) the first visible myeloid body. Fixative: 2% glutaraldehyde in seawater. Scale bar = 100 μm.

FIGURE 5. TEM of a sagittal section through outermost tips of the receptors. Supporting cell processes are still clearly visible. Fixative: 2% glutaraldehyde in phosphate buffer. Scale bar = 5 μm.







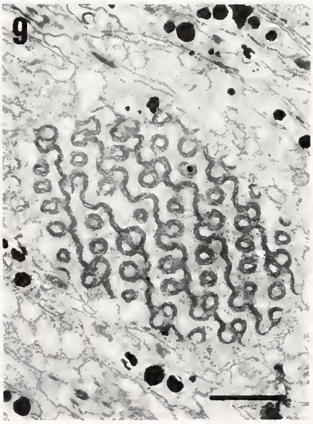


FIGURE 6. TEM of a sagittal section through the outer segments at the transitional zone at the edge of the retina. The iris is to the right. Fixative: 2% glutaraldehyde in seawater. Scale bar = 2 μm.

FIGURE 7. TEM of sagittal section through the inner segments of retinal receptors showing the relative positions of nuclei and myeloid bodies. The bottom of the photograph is internal. Fixative: 2% glutaraldehyde in seawater. Scale bar = 10 μm.

FIGURE 8. TEM of a sagittal section through the outer segments of the retina showing displaced myeloid body. Microtubules and supporting cell processes are clearly visible. Fixative: 2% glutaraldehyde in phosphate buffer. Scale bar = 1 μm.

FIGURE 9. TEM of a sagittal section of a myeloid body showing membranous structure. Fixative: 2% glutaraldehyde in phosphate buffer. Scale bar = 2 μm.

been reported in the retina of any adult cephalopod. The photosensitive organs of many animals have receptors of ciliary origin, or cilia, presumed not to be photosensitive, intermingled with the receptors (Vanfleteren, 1982). Many rhabdomeric receptors possess cilia in their embryonic or larval stages but not in the adult stage (Vanfleteren, 1982). Yamamoto (1985) reported that such structures are present in both the receptors and supporting cells of the embryos of the cuttlefish, *Sepiella japonica*, persisting longer in the supporting cells than in the receptors. The retina of the *S. japonica* embryo resembles that of *Nautilus* in a number of other respects. These include the fact that in early stages the nuclei of both supporting and receptor cells are external to the basal membrane, whereas in adults only the supporting cell nuclei remain external; that the microvilli of the receptor cells occur initially more or less equally in all directions, only achieving the regular rectilinear arrangement of adults at a relatively late stage of development; and that the supporting cells in embryos send long microvillous processes between the receptor cells over the whole length of their outer segments. They also possess cilia.

The myeloid bodies of *Nautilus*, which are found in the inner segments of the receptors, are much more complex than those found in the coleoid cephalopods. Barber and Wright (1969) state that they are usually found externally to the nucleus, while Merton's (1905) figures show them equally on either side of the nucleus. In our preparations they lay internally to the nucleus more often than externally, and they were also on occasion found displaced into the outer segment.

Myeloid bodies did not occur at the retinal margin, but only some 100–200 μm inwards after the point at which the retina outer layer starts to lengthen. The edge of the retina also contains relatively few rhabdomeres and many supporting cells. The presence of the myeloids may thus correlate with the presence of rhabdomeres.

The structure of the myeloid bodies has been described from light microscope studies as a stacked series of cubes, or a honeycomb structure (Merton, 1905), and from electron micrographs as a complex tubular array (Barber and Wright, 1969). Both Barber and Wright's electron microscope results and those shown in Figures 7, 8, and 9 of the present paper appear to confirm both the complexity of and on many occasions an apparently tubular structure. However, the photographs often give the impression of a series of stacked wavy plates, or of a structure where both forms of organization alternate or merge.

Despite the apparent complexity, there is a comparatively simple structure that can generate most or all of these different appearances depending on the plane of section, namely a series of dimpled plates stacked in register one above the other. Mathematically the surface of each individual plate can be approximated by the relationship $z = \cos x \cos y$ (Fig. 10). A section in the plane of the plates will clearly produce a pattern of circles, squared off to a greater or lesser extent depending on how close the section is to the point where $z = 0$, which could be interpreted as a tubular structure. Similarly, a section perpendicular to the plane of the plates will produce an appearance similar to a stacked series of wavy plates, the amplitude of the waves depending on how close the section is to the lines defined by $\cos x$ or $\cos y = 0$. Various intermediate patterns similar to those seen in the electron micrographs are generated when the section cuts the plate at an angle (see examples in Fig. 11). Of course these patterns will be repeated with a frequency which will depend on the spacing between the plates constituting the stack. The individual plates, or the whole stack, could also be curved, which would affect the appearance of the myeloid bodies.

The function of myeloid bodies remains uncertain. However the available evidence shows that in other cephalopods retinochrome is found in association with the myeloid bodies, and that the distribution of retinoids between the myeloid bodies

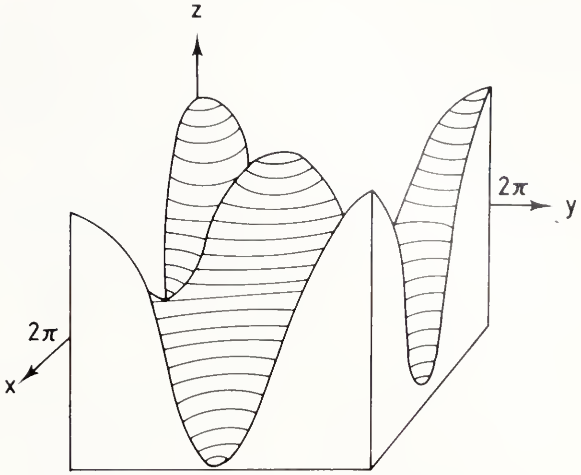


FIGURE 10A. Suggested structure of myeloid bodies. Appearance of a surface defined by cos x cos y = z.

and the outer segments of the receptors can be affected by the light regime under which the animals are kept (Hara and Hara, 1972; Breneman *et al.*, 1986). Thus the myeloid bodies are implicated in the visual pigment metabolism of cephalopods, most likely being concerned with pigment regeneration. Metabolic events often occur at the surfaces of membranes and thus depend on the membrane surface area. A stack of dimpled plates of the sort proposed represents a simple way of increasing the total surface area of membrane present in the myeloid body without increasing its gross dimensions. Why *Nautilus* should have a myeloid body that is so structurally complicated compared to other cephalopods remains unresolved.

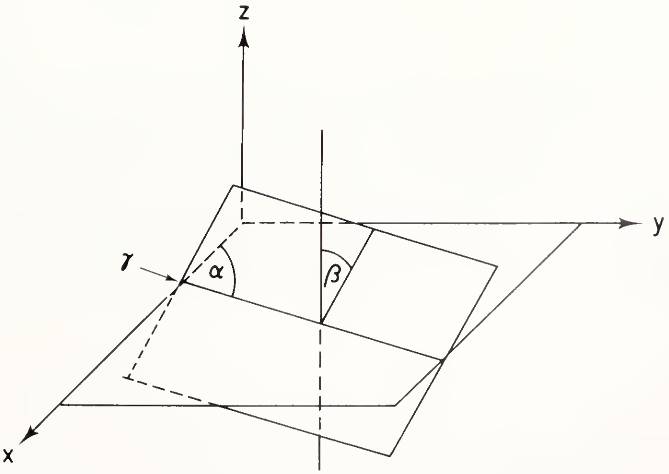


FIGURE 10B. Suggested structure of myeloid bodies. Any plane of section of the surface x, y can be defined by the two angles α and β and by the value of γ.

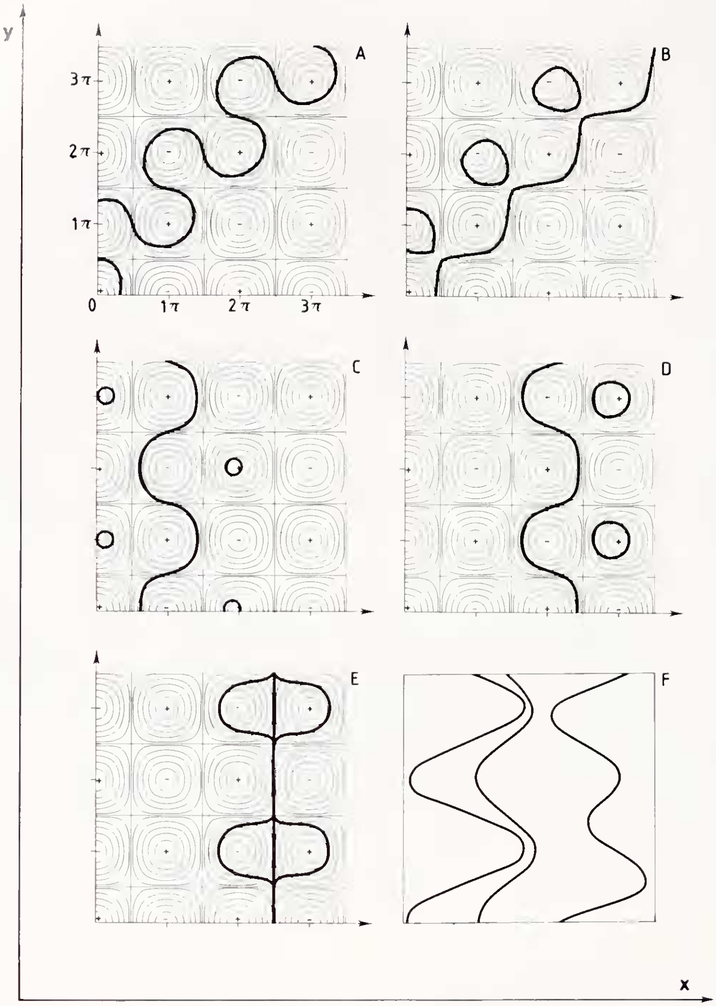


FIGURE 11. A to E, contour maps of the surface shown in Figure 10A, with the appearance of the cut surfaces that would be produced by sections cut in planes defined by different values of α and γ, β having a fixed value of π and z a maximum of 1 in all cases. The contour lines show heights and depths above and below the plane of the surface for values of 0, 0.2, 0.4, 0.6, and 0.8 of the maximum. A; $\alpha = 0.25\pi$, $\gamma = 1.5\pi$. B; $\alpha = 0.25\pi$, $\gamma = 0$. C; $\alpha = 0.5\pi$, $\gamma = \pi$. D; $\alpha = 0.5\pi$, $\gamma = 0.25\pi$. E; $\alpha = 0.5\pi$, $\gamma = 0.5\pi$. F, appearance of sections perpendicular to the plate (i.e. $\beta = 90°$), for values of α of 0.5π and values of γ from left to right of $0.5\ \pi$, 0.25π, and 1.75π respectively. Superimposed on a contour map such sections would appear as straight lines: they are shown here as they would be seen from a position perpendicular to the plane of section.

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